One of the protein components of lens fiber membranes is glyceraldehyde 3-phosphate dehydrogenase

Johannes A. Lenstra, Adriana J.M. van Raaij and Hans Bloemendal

Department of Biochemistry, University of Nijmegen, Geert Grooteplein Noord 21, 6525 EZ Nijmegen, The Netherlands

Received 6 September 1982

The eye lens membrane component 'MP34' [Exp. Eye Res. 24 (1977) 413–415] has been resolved into three protein components and in a revised nomenclature designated MP35, MP36.5 and MP37, respectively. MP37 has been identified as the enzyme glyceraldehyde 3-phosphate dehydrogenase, which is one of the major components of membranes both from cultured hamster lens cells and from HeLa cells.

Eye lens *Glyceraldehyde 3-phosphate dehydrogenase* *Membrane protein* *Peptide mapping* *Two-dimensional gel electrophoresis* *pp60^{src}*

1. INTRODUCTION

There has been a growing interest in the morphology and biochemistry of lens fiber cell membranes [1,2]. There are strong indications that these plasma membranes interact with at least some of the structural lens proteins, the crystallins [3], filamentous actin [2] and vimentin [4]. However, the nature of these interactions has not been elucidated, being hampered by insufficient knowledge of the protein components of lens membranes.

Lens plasma membranes purified by flotation have a relatively simple pattern in which a M_r 26 000 component represents ~50% of the total protein, while another pronounced 'component' is found in the M_r 34 000–35 000 region after SDS–polyacrylamide gel electrophoresis [5,6]. About the same polypeptide pattern is obtained with membranes, isolated as the urea-insoluble fraction of lens homogenates [6,7]. Extraction of this preparation by EDTA liberated the M_r 34 000–35 000 protein, or EEP (EDTA-extractable protein). Gel electrophoresis suggested that EEP consists of two components with app. M_r 32 000 and 35 000, respectively [7].

We wish to report here that the M_r 35 000 component from purified lens membranes consists of 3 unrelated polypeptides, one of which is identical with glyceraldehyde 3-phosphate dehydrogenase. One of the other polypeptides is present not only in lens fiber membranes, but also, as the major component, in the membranes from cultured hamster lens epithelial cells [8,9] as well as from HeLa cells.

2. MATERIALS AND METHODS

Glyceraldehyde 3-phosphate dehydrogenase from rabbit muscle was purchased from Boehringer (Mannheim) as a crystal suspension, spun down and dissolved in SDS containing sample buffer [10]. Bovine lens fiber membranes were isolated as in [11] as interface after discontinuous gradient centrifugation with a density of 1.14–1.18 g/ml. Membranes from hamster lens epithelial cells, transformed by SV40 and grown in suspension culture [8], and from HeLa cells were isolated as in [12]. Urea-insoluble lens proteins (UIL) and EDTA-extractable protein (EEP) were isolated as in [7].

Enzymes: glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12)

Abbreviations: G3PD, glyceraldehyde 3-phosphate dehydrogenase; EEP, EDTA-extractable protein; UIL, ureum-insoluble fraction of lens homogenate

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

SDS–polyacrylamide gel electrophoresis in slabs 0.7 mm thick was performed as in [10]. Two-dimensional gel electrophoresis after non-equilibrium pH-gradient gel electrophoresis (Ampholines pH 3.5–10 from LKB, Uppsala, 800 V.h) and one-dimensional peptide mapping was done as in [13] and [14], respectively, with some simplifications [12]. Prior to electrophoresis, membrane preparations were heated in sample buffer at 60°C for 10 min, conditions which did not result in aggregation of MP26.

3. RESULTS AND DISCUSSION

Fig.1 shows gel electrophoretic profiles on thin-polyacrylamide slabs of the membrane preparations used in this study. In calf lens membranes (fig. 1B, CL), 3 components of M_r 35 000–37 000 can be resolved. On the basis of their M_r-values, we designate these components as MP37, MP36.5 and MP35, respectively, replacing the designation 'MP34' for the broad protein band originally observed [1,5,6,15]. Likewise, two other components are designated MP33 and MP32.5, respectively. MP37 and MP36.5 migrate as the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (fig.1A, G3PD), which is one of the major components (band 6) of erythrocyte membranes [16]. MP35 has the same mobility as the major membrane component from cultured hamster lens cells (fig.1C).

In contrast to the major lens membrane protein, MP26, the components in the M_r 30 000–37 000 range are not degraded as a consequence of storage of the sample (fig.1D, CL′; cf. [15]). Preparations of calf lens membranes as urea-insoluble fraction (fig.1E, UIL) yields about the same components as membranes isolated by flotation with the notable exception, however, that MP37 is absent. Treatment of UIL with EDTA (fig.1F, EEP) liberates MP36.5, MP35, MP33, MP32.5 and a component designated MP31. Most probably, the described M_r 35 000 and 32 000 components of EEP [7] consist of MP36.5 plus MP35 and MP33, MP32.5 plus MP31, respectively.

The M_r 35 000 membrane component of cultured lens cells (fig.1B and G, HLSV) is also present in HeLa cell membranes (fig.1H). This was confirmed by two-dimensional gel electrophoresis [12].

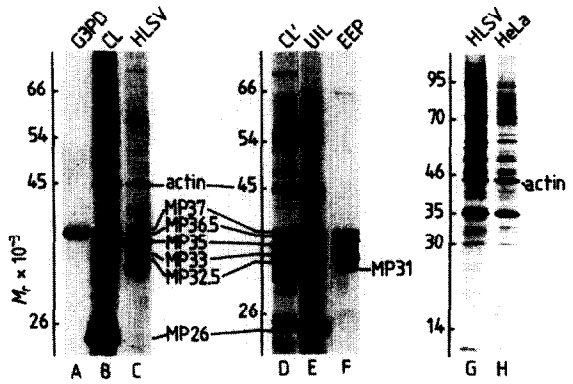

Fig.1. Gel electrophoretic profiles of: (A, G3PD) 1 μg rabbit muscle glyceraldehyde 3-phosphate dehydrogenase; (B, CL) calf lens membranes purified by flotation, 35 μg protein; (C, HLSV) membranes from cultured hamster lens cells, 16.5 μg protein; (D, CL′) calf lens membranes after storage at – 20°C; (E, UIL) calf lens membranes isolated as urea-insoluble fraction of lens homogenate; (F, EEP) EDTA-extractable proteins from UIL; (G, HLSV) as (B, HLSV); (H, HeLa) HeLa cell membranes. Lanes (D–F) were from the same slab gel; (A–F) proteins separated in 10% (w/v) polyacrylamide were stained with Coomassie brilliant blue; (G,H) proteins labelled in vivo with [^{35}S]methionine and separated in 7–18% (w/v) polyacrylamide gradient gels were detected by autoradiography. MP26 denotes the major intrinsic membrane protein from calf lenses; A′ indicates the position of actin in membranes from cultured cells.

In order to elucidate if MP37, MP36.5, MP35, the band 6-protein from erythrocyte membranes (glyceraldehyde 3-phosphate dehydrogenase) and the M_r 35 000 major membrane component from cultured cells are related, these proteins were isolated by excising gel bands and subjected to partial proteolytic cleavage (fig.2). Although the bands of MP37 and MP36.5 were cross-contaminated, it is clear that there is no apparent relationship between the 3 lens membrane proteins. However, MP37 has the same cleavage pattern as glyceraldehyde 3-phosphate dehydrogenase (2 major bands; the M_r 20 000 minor band of G3PD is just detectable with MP37), while MP35 is identical to the major membrane protein from cultured cells.

The identification of MP37 as glyceraldehyde 3-phosphate dehydrogenase could be confirmed by two-dimensional gel electrophoresis. While after

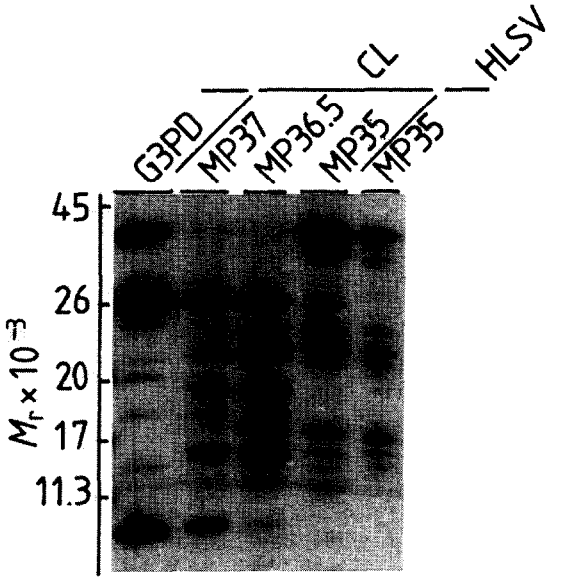

Fig.2. Peptide profiles of membrane proteins after cleavage with *Staphylococcus aureus* V8 protease. G3PD, rabbit muscle glyceraldehyde 3-phosphate dehydrogenase; CL, calf lens membranes; HLSV, membranes from cultured hamster lens cells. MP37, MP36.5, MP35, membrane proteins with app. M_r 37 000, 36 500 and 35 000, respectively. Peptides were separated by electrophoresis in 18% (w/v) polyacrylamide and detected with Coomassie brilliant blue. Amounts of protease and membrane proteins were: 50 ng protease with 5 μg G3PD; 50 ng, 50 ng and 10 ng protease with 15 times the amounts of CL MP37, -36.5 and -35, respectively, used for fig.1B; 10 ng protease with 2.5-times the amount of HLSV MP35 used for fig.1C.

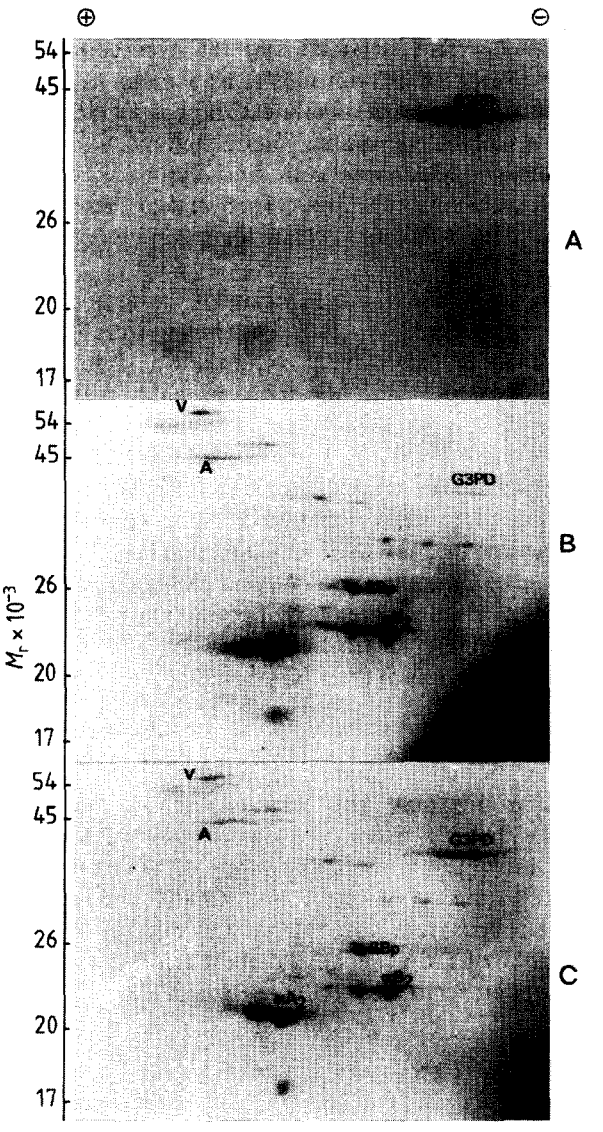

Fig.3. Two-dimensional gels of: (A) 8 μg glyceraldehyde 3-phosphate dehydrogenase; (B) calf lens membranes, 170 μg protein; (C) 170 μg protein, plus 4 μg glyceraldehyde 3-phosphate dehydrogenase. After non-equilibrium pH-gradient electrophoresis in the horizontal direction (origin at the left side), proteins were separated by electrophoresis in 13% (w/v) polyacrylamide gels. αA2, αB2, βBp, A and V denote the corresponding lens crystallins, actin and vimentin, respectively.

equilibrium isoelectric focusing in the first direction MP37 could not be detected on gel patterns of lens membranes (not shown), after non-equilibrium focusing (fig.3B) one M_r 37 000 spot is observed, which is in the same position as the spot of G3PD (fig.3A). During co-electrophoresis (fig.3C), G3PD and the lens membrane component comigrate completely.

Glyceraldehyde 3-phosphate dehydrogenase is loosely associated with erythrocyte membranes, both in vivo and in vitro [16]. Our results indicate that the same is the case in lens fiber membranes. The absence of the enzyme from urea-extracted lens membranes also indicates a loose association. As the functional significance of the membrane association of G3PD (near the transmembrane anion-transport protein) is unknown in the case of erythrocytes [16], the same holds true for lens fiber

membranes. Membranes from other cell types, as cultured cells (fig.1G,H) and rat liver cells (not shown) do not contain G3PD as a major component. We suggest that the membrane association of this glycolytic enzyme may be related to a common feature of lens fiber cells and eythrocytes: the absence of mitochondria, which makes these cells dependent on the glycolysis for their energy production.

At the moment, the function of the major protein component of membranes from cultured cells, identical to lens MP35, is unknown. Published peptide maps of the M_r 36 000 probable substrate of pp60^{src} from Rous sarcoma virus, with 32, 24, 23, 19 and 18 $\times$ 10^{-3} M_r bands [17], bear some similarity to the maps of MP35 in fig.2, but it is not warranted to draw conclusions upon comparison of peptide maps obtained by different laboratories. Yet analysis of proteins, detected as bands or spots after gel electrophoresis, by peptide mapping may be an easy way to their identification and helpful with elucidation of their function.

ADDENDUM

After finishing this manuscript we became aware that Dr Jedziniak (Massachusetts Eye and Ear Infirmary Howe Laboratory of Ophthalmology, Boston MA) has found G3PD activity in human lens removable from the plasma membranes by EDTA (personal communication to H.B. and report 65 to the ARVO meeting 1982).

REFERENCES

[1] Benedetti, E.L. (1982) in: Molecular and Cellular Biology of the Eye Lens (Bloemendal, H. ed) pp. 137–188, Wiley, New York.

[2] Bloemendal, H. (1982) CRC Crit. Rev. Biochem. 12, 1–39.

[3] Bloemendal, H., Hermsen, T., Dunia, I. and Benedetti, E.L. (1982) Exp. Eye Res. 35, 61–67.

[4] Ramaekers, F.C.S., Dunia, I., Dodemont, H.J., Benedetti, E.L. and Bloemendal, H. (1982) Proc. Natl. Acad. Sci. USA 79, 3208–3212.

[5] Vermorken, A.J.M., Hilderink, J.H.M.C., Dunia, I., Benedetti, E.L. and Bloemendal (1977) FEBS Lett. 83, 301–306.

[6] Benedetti, E.L., Dunia, I., Bentzel, C.J., Vermorken, A.J.M., Kibbelaar, M. and Bloemendal, H. (1976) Biochim. Biophys. Acta 457, 353–384.

[7] Bouman, A.M., De Leeuw, A.L.M., Tolhuizen, E.F.J. and Broekhuyse, R.M. (1979) Exp. Eye Res. 29, 89–93.

[8] Bloemendal, H., Lenstra, J.A., Dodemont, H., Ramaekers, F.C.S., Groeneveld, A., Dunia, I. and Benedetti, E.L. (1980) Exp. Eye Res. 31, 513–525.

[9] Lenstra, J.A., Hukkelhoven, M.W.A.C., Groeneveld, A.A., Smits, R.A.M.M., Weterings, P.J.J.M. and Bloemendal, H. (1982) Exp. Eye Res. in press.

[10] Laemmli, U.K. and Favre, M. (1973) J. Mol. Biol. 80, 575–599.

[11] Bloemendal, H., Zweers, A., Vermorken, F., Dunia, I. and Benedetti, E.L. (1972) Cell Diff. 1, 91–106.

[12] Lenstra, J.A. and Bloemendal, H. (1982) submitted.

[13] O'Farrell, P.Z., Goodman, H.M. and O'Farrell, P.H. (1977) Cell 12, 1133–1142.

[14] Cleveland, D.W., Fischer, S.G., Kirschner, M.W. and Laemmli, U.K. (1977) J. Biol. Chem. 251, 1102–1106.

[15] Bloemendal, H., Vermorken, A.J.M., Kibbelaar, M., Dunia, I. and Benedetti, E.L. (1977) Exp. Eye Res. 24, 413–415.

[16] Gillies, R.J. (1982) Trends Biochem. Sci. 7, 41–42.

[17] Radke, K., Gilmore, T. and Martia, G.S. (1980) Cell 21, 821–828.